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LIPID-PARTITIONING AND DISPOSITION OF BENZO[*a*]PYRENE AND HEXACHLOROBIPHENYL IN LAKE MICHIGAN *PONTOPOREIA HOYI* AND *MYSIS RELICTA*

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Abstract—Two Lake Michigan macroinvertebrates, *Pontoporeia hoyi* and *Mysis relicta*, exhibited major differences in the disposition of the lipophilic contaminants, [^{3}H]benzo[*a*]pyrene (BaP) and [^{14}C]2,4,5,2′,4′,5′-hexachlorobiphenyl (HCB). Interactions of these contaminants with major lipid classes (triacylglycerols and phospholipids) were examined by centrifuging aqueous whole organism homogenates of labeled animals into three discrete layers that were operationally defined as "buoyant-lipid," "particle" and "aqueous" fractions. The buoyant-lipid fraction contained most of the energy storing triacylglycerols, whereas the particle and aqueous fractions contained most of the membrane phospholipids. During 2 to 4 d experiments, unmodified BaP and HCB partitioned among the three fractions in proportion to the distribution of total-lipids in both species, but in *M. relicta* most of the BaP was biotransformed into polar metabolites that were selectively found in the aqueous and particle fractions. Apparently HCB was not substantially biotransformed in either species but took longer (ca. 2 d) to reach steady state among lipid pools in *M. relicta* than it did in *P. hoyi* (<1 d). Although the contaminants did not always completely reach steady state in the organisms with respect to the external environments during these relatively short experiments, they appeared to reach steady state among lipid pools within the organisms.

Keywords—Lipids Contaminants Invertebrates *Pontoporeia* *Mysis*

INTRODUCTION

Hydrophobic xenobiotics that are not metabolized generally accumulate in aquatic organisms in proportion to total-lipid content [1,2]. The transfer of these compounds through aquatic food webs is therefore thought to be affected by their interactions with lipids [3]. In the upper Great Lakes, the macroinvertebrates *Pontoporeia hoyi* and *Mysis relicta* contain relatively high levels of lipids [4] and, as important prey, both are thought to transfer organic contaminants to fish [5–7]. *P. hoyi* is a benthic amphipod commonly eaten by *M. relicta* [8], bloater-chubs, sculpins and whitefish, as well as by alewives, whereas *M. relicta* is a vertically migrating malacrustacean eaten primarily by alewives, smelt and lake trout in Lake Michigan (S.W. Hewitt, personal communication). Although these two marine glacial-relict species have comparable total-lipid levels and large bioconcentration factors for nonpolar organic contaminants, their disposition of the contaminants (i.e., the overall dynamics of the compounds within the organism, including uptake, biotransformation and elimination) appears to be different. For example, *P. hoyi* eliminates organic contaminants (e.g., benzo[*a*]pyrene) slowly and the rate of elimination is inversely related to the total lipid content [9]. However, in *M. relicta* the relationship between lipid content and elimination of [^{3}H]benzo[*a*]pyrene (BaP) is not as direct [10]. Such differences in metabolic strategies of major species may affect the ultimate fate of the contaminants in aquatic ecosystems. For example, based on the above observations, *P. hoyi* could be expected to pass more of its assimilated BaP to its predators than would *M. relicta*. Thus, even if both species were exposed to equal amounts of BaP in the environment, fish that selectively feed on *P. hoyi* over *M. relicta* would be exposed to more BaP in their food than those that ingest equal amounts of the two macroinvertebrates or are selective for *M. relicta*.

Note that some of the differences in contaminant dynamics between these two species could result from dissimilar interactions between lipid

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classes and contaminants. If the contaminants were associated strongly with energy storage lipids, then reduction in the lipid pool due to depletion of energy reserves should cause increased elimination of the compound from that pool. Almost no information is available on specific interactions between contaminants and the different classes of lipids in invertebrates. It is also unclear how organic contaminants in organisms are distributed among the lipid classes, such as energy storage lipids (e.g., triacylglycerols or wax esters) or structural lipids (e.g., phospholipids). Differential associations of contaminants with major lipid classes have been examined in fish tissues [11–13], but results differed among investigations depending on the solvents used for the extractions. Bound phospholipids, and associated contaminants, are inefficiently extracted from the tissue particles with nonpolar solvents [12,13]. A relatively polar solvent combination (such as chloroform–methanol) is needed to quantitatively extract structural as well as free lipids.

The issue of how organic contaminants may associate with the different lipid classes in organisms is difficult to address because when the lipids and contaminants are coextracted with an organic solvent that effectively dissolves both components, the extraction process may cause the contaminant to dissociate from the lipid component that held it in the animal. To overcome this problem, we physically separated storage lipids from the structural lipids before solvent extraction. Potential lipid-contaminant associations in the benthic macroinvertebrates, *P. hoyi* and *M. relicta*, were examined by exposing the animals to the labeled contaminants, BaP and [^{14}C]2,4,5,2′,4′,5′-hexachlorobiphenyl (HCB), and then by fractionating homogenates of the animals into buoyant-lipid, aqueous and particle fractions. Each fraction was then either analyzed directly for radiolabels or extracted with chloroform–methanol, and analyzed for lipid-classes, to provide insights about the dynamics of the contaminants, with respect to lipids, in the respective organisms.

METHODS

Macroinvertebrate collection and contaminant exposure

Pontoporeia hoyi (ca. 6–8 mg wet weight animal^{-1}) were collected in August and September from Lake Michigan approximately 2 miles west of Grand Haven, Michigan in 30 m of water with a PONAR sampler. The animals were gently screened from the sediments, placed in hypolimnetic lake water, transported to the laboratory in coolers on ice and then either processed within 48 h (August experiment) or held at 4°C for about three weeks (September experiment) in plexiglass aquaria containing 2 to 4 cm of lake sediment and 10 cm of lake water under a red darkroom light. *P. hoyi* can be stored for several months under these conditions with minimal mortality (unpublished observations).

Mysis relicta (ca. 40–100 mg wet weight animal^{-1}) were sampled in Lake Michigan approximately 7 miles west of Grant Haven, Michigan in 80 to 100 m of water in September and November, 1988. *M. relicta* were collected at night, to coincide with their nocturnal vertical migration [14], by towing a Nitex net (1 m × 3 m; 561 μm mesh) slowly 2 to 3 m off the lake bottom. The animals were transferred to 50 liters of lake water maintained at less than 10°C and returned to the laboratory. They were housed in plexiglass aquaria containing aerated lake water (but no sediments) at 4°C in the dark for 1 to 3 d until the experiments were begun.

For contaminant exposure experiments, the animals were simultaneously exposed to [^{14}C]2,4,5,2′,4′,5′-hexachlorobiphenyl (HCB) (14.06 mCi mmol^{-1}) and [^{3}H]benzo[*a*]pyrene (BaP) (23.8 Ci mmol^{-1}). The labeled compounds were examined for radiopurity by thin layer chromatography using hexane:benzene (8:2 v:v) as the solvent system and liquid scintillation counting for detection [15]. The required purity for use was 98% or greater. The compounds were dosed into lake water that had been filtered through glass-fiber filters (Gelman AE; nominal pore size of 1 μm). Up to 6 liters of lake water were dosed with the radiotracer in a methanol carrier (<10 mg methanol L^{-1}). The solution was thoroughly mixed, distributed between two 6-liter glass aquaria (4 liters in the *M. relicta* aquarium and 2 liters in the *P. hoyi* aquarium), and allowed to equilibrate for 1 h prior to adding the organisms. The contaminant concentrations in the water for the different experiments ranged from 1.1 to 2.4 pg ml^{-1} for BaP and 3.0 to 5.9 ng ml^{-1} for HCB at the beginning of the exposures. *M. relicta* (about 20 individuals) and *P. hoyi* (about 60 individuals) were added to the respective aquaria and then removed for analysis after exposures of 24 to 96 h. Note, because the exposures were static, the concentrations in the water decreased over time. For example, in the November experiment on *M. relicta*, measured concentrations of BaP and HCB declined 74% and 91%, respectively, during the 4-d exposures. Final exposure concentrations were not measured in the earlier experiments. Al-

though total flux into the organisms would decline with decreasing water concentrations (or increased organism loading), this decline would not be expected to affect the relative distributions of the contaminants among lipid pools in the organisms. Replicate measurements were made on respective individuals or groups of animals from a given exposure, but exposures were not replicated in separate aquaria within a given experiment. Additional aquaria with filtered lake water but no radiotracers were set up to provide organisms for lipid analysis. Samples for lipid analysis were removed at about 50 h. Lipid class distributions were assumed to remain constant over this relatively short period as lipid levels in *P. hoyi*, and presumably *M. relicta*, do not significantly change during the first few days after they are removed from food (unpublished data).

Physical fractionation and measurement of lipids and contaminants

The glass barrel of a Wiretrol pipet (Drummond Scientific Co., Broomall, PA) was modified into a special centrifuge tube by drawing it out with a flame at the 100-μl mark (Fig. 1a–d). The macroinvertebrates (three *P. hoyi* or one *M. relicta*) were blotted dry with tissue and placed in a test tube (6 mm o.d. × 50 mm length). About 90 μl of 0.9% NaCl in distilled water were added and the animals were homogenized for 15 s with a microtissue homogenizer constructed from a Rotary tool with a Teflon–glass pestle [16]. The homogenate was vortexed and most of it was drawn into the lower portion of the drawn out pipet barrel (Fig. 1c) using for suction a modified (tip removed) plastic syringe (0.5-ml insulin syringe equipped with a septum, illustrated in upper portion of Fig. 1e). The lower end of the barrel was then plugged with a moist preparation of *inorganic* potter's clay (obtained from an art supply store). The upper section of the drawn out pipet, that was attached to the syringe, was broken off (at about 75 mm above the lower clay plugged end) and discarded. Additional clay was pressed into the lower tube, to push the surface of the homogenate up into the constricted part of the tube, and the tube was spun (at 13,500 *g*) in a hematocrit centrifuge (IEC MB microhematocrit centrifuge) for 2 min. This centrifugation partitioned the homogenate into three layers (Fig. 1d). The upper buoyant-lipid layer was extruded into one receiving vessel by gently pushing the Wiretrol pipet plunger against the clay layer at the bottom of the tube. The constricted end of the tube was then inserted into an open septum attached to the syringe (with the plunger drawn), to prevent loss of the aqueous layer when the tube was cut, and the tube was scored and broken at the liquid–particle interface (Fig. 1e). The particle layer was extruded into a second receiving vessel from the lower portion of the tube by pushing the clay layer further up the tube with the Wiretrol plunger. The particle plug was easily dislodged from the clay by touching it to the side or bottom of the receiving vessel. Finally, the aqueous layer was injected into a third receiving vessel by pushing down the plunger of the attached syringe.

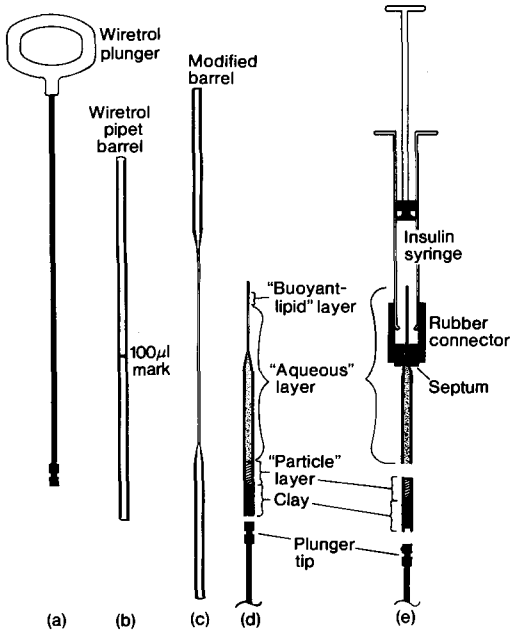

Fig. 1. Schematic diagram of Wiretrol system modified to physically fractionate lipids in aquatic invertebrates into three fractions. The width-to-length ratios of the pipets are exaggerated on the diagram for illustrative purposes.

Tared test tubes (6 mm o.d. × 50 mm length), used as grinding tubes in the lipid extraction procedure [16], were the receiving vessels for the different fractions collected for lipid-class analysis. After partitioning, the residues of each phase were dried under a slow flow of nitrogen gas in a dessicator held at about 50°C and then stored frozen under nitrogen in a dessicator until lipid extraction and analysis. Lipids were extracted with chloroform:methanol (2:1 v/v), washed with an aqueous salt solution, and quantified gravimetrically using a microversion [16] of the Folch et al. [17] method for total lipid analysis. Lipid classes were deter-

mined on a portion of the extracts by thin-layer chromatography with flame ionization detection (TLC-FID) [18–20].

To determine the radiotracer activity, the three partitioned phases were placed in respective scintillation vials with cocktail (3a70B Research Products International) for counting. The samples were counted on an LKB 1217 scintillation counter and corrected for quench by the external standards ratio method after subtracting background. Fractions of additional *M. relicta* samples from the November experiment were extracted and preserved in ethyl acetate. These samples were concentrated under nitrogen and analyzed by thin-layer chromatography (TLC) to separate parent compounds from polar metabolites [21] in each of the fractions. After development with hexane:benzene (8:2, v/v), sections from the thin-layer plates were transferred to scintillation vials and analyzed for radiotracer activity. Unlabeled BaP was used as a standard to visualize the extent of migration of the parent compound.

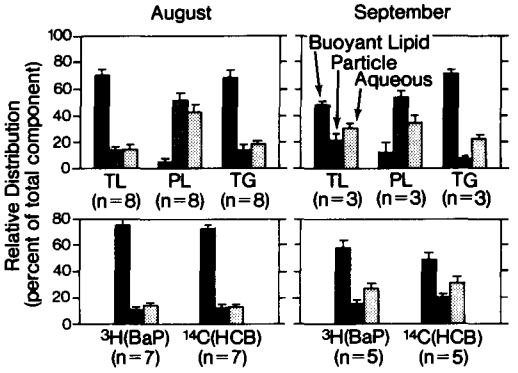

Fig. 2. Relative distributions of total lipids (TL), triacylglycerols (TG), phospholipids (PL), BaP and HCB among the three physically-separated fractions in *P. hoyi*, after 2-d exposures, for experiments begun on 24 August and 29 September 1988. Each bar represents a mean value for measurements on replicate groups of animals. Each vertical line with a horizontal hat represents one SE. *n* = number of replicate measurements.

RESULTS

Lipid distributions

Partitioning of total lipids among the three fractions was generally similar for the two species. About 50 to 70% of the lipids in both *P. hoyi* (Fig. 2) and *M. relicta* (Fig. 3) were found in the buoyant-lipid fraction collected from the top of the supernatent after centrifugation. The remaining lipids were distributed approximately evenly between the particle and aqueous fractions but in some samples the aqueous fractions contained significantly more total lipids than did the particle fractions (Figs. 2 and 3). Lipid-class analysis of the respective fractions indicated that triacylglycerols and phospholipids together accounted for more than 80% of the total lipids measured in both *P. hoyi* and *M. relicta* (Tables 1 and 2). Triacylglycerols constituted more than 85% of total lipids in the buoyant-lipid fraction for both species. Phospholipids and triacylglycerols were the major lipid components of both the aqueous and particle fractions. These fractions contained more than 75% of the total phospholipids but only about 10 to 45% of the triacylglycerols (Tables 1 and 2). However, because the animals contained more total triacylglycerols than phospholipids, the relative concentrations of triacylglycerols in the aqueous and particle fractions sometimes resembled those of phospholipids (Tables 1 and 2). Relative percentages of other classes of lipids were very small in these two fractions except for the aqueous fraction of *P. hoyi* in the September experiment that consistently contained measurable quantities of free fatty acids (FFA), sterols and acetone-mobile polar lipids (AMPL) (Table 1). All fractions in both animals contained small amounts (0.3–3% of the total lipids) of sterols and AMPL (Tables 1 and 2).

Xenobiotic distributions

Although total amounts of contaminant accumulated by *P. hoyi* increased with time of exposure

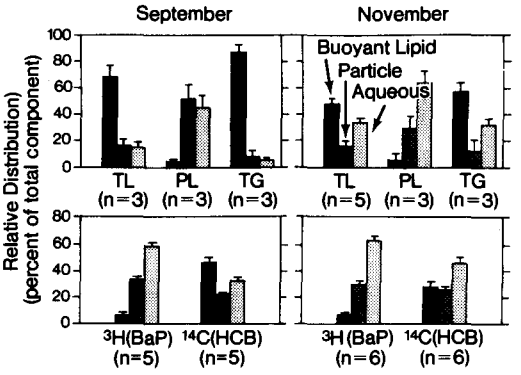

Fig. 3. Relative distributions of total lipids (TL), triacylglycerols (TG), phospholipids (PL), BaP and HCB among the three physically-separated fractions in *M. relicta*, after 2-d exposures, for experiments begun on 29 September and 9 November 1988. Each bar represents a mean value for measurements on replicate animals. Each vertical line with a horizontal hat represents one SE. *n* = number of replicate measurements.

Table 1. Relative distributions of the major lipid classes in the buoyant-lipid, particle and aqueous fractions in *P. hoyi* expressed as percent of total TLC-FID lipids in the three fractions from the same animals

	August experiment ($n = 8$)						September experiment ($n = 3$)					
	Buoyant-lipid		Particle		Aqueous		Buoyant-lipid		Particle		Aqueous	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
TG	52.5	4.9	9.6	2.8	13.6	2.3	38.5	8.6	3.6	0.5	11.4	1.7
FFA	nd	–	1.2	0.4	1.3	1.1	nd	–	1.4	0.2	9.1	1.8
ALC	0.5	0.2	nd	–	nd	–	nd	–	nd	–	nd	–
ST	0.5	0.1	0.9	0.1	1.0	0.2	1.2	0	1.1	0.2	2.1	0.2
AMPL	1.4	0.1	0.6	0.1	1.1	0.2	1.3	0.1	0.7	0.3	1.6	0.1
PL	0.9	0.4	8.3	2.1	6.3	1.3	2.8	1.3	15.6	5.6	9.4	2.8

TG = triacylglycerols; FFA = free fatty acids; ALC = aliphatic alcohols; ST = sterols; DG = diacylglycerols; AMPL = acetone mobile polar lipids; PL = phospholipids; nd = not detected.

during the first 2 d (e.g., Fig. 4), relative distributions of ^{3}H (from BaP) and ^{14}C (from HCB) among the three fractions in *P. hoyi* did not vary between the first and second day (Table 3). The similarity of percent-distribution results between day 1 and day 2 for the two compounds in *P. hoyi* suggests that the contaminant distributions had likely approached steady state among the various lipid classes *within* this species after 1 d of exposure. Likewise, the results for BaP in *M. relicta* (Table 3) were consistent after the first day. However, the percentage of HCB in the buoyant-lipid fraction of *M. relicta* increased from 23% on day 1 to 46% on day 2 in the September experiment and from 8 to 27% in the November experiment. Contaminant distributions did not change significantly between days 2 and 4 (Table 3).

The distribution patterns of both ^{3}H (from BaP) and ^{14}C (from HCB) followed that of total lipids in *P. hoyi* on both days (Fig. 2). In *M. relicta*, ^{14}C tended to be enriched in the aqueous fraction and depleted in the buoyant-lipid fraction relative to the distributions of total lipids (Fig. 3). The distribution pattern of ^{3}H in *M. relicta* was different from that of ^{14}C, or from that of either of the isotopes in *P. hoyi*. As was true for the distribution of phospholipids, only a small portion (7%) of the total ^{3}H from BaP partitioned into the buoyant-lipid fraction. These results suggest that, in contrast to *P. hoyi*, *M. relicta* partitions BaP (or BaP metabolites) differently than HCB among lipid classes. The difference did not appear to be a function of equilibration-time because the BaP results for day 2 (and day 4) were almost identical to those for day 1 (Table 3).

In the November experiment, some *M. relicta*

Table 2. Relative distributions of the main lipid classes in the buoyant-lipid, particle and aqueous fractions in *M. relicta* expressed as percent of total TLC-FID lipids in the three fractions from the same animal

	September experiment ($n = 3$)						November experiment ($n = 3$)					
	Buoyant-lipid		Particle		Aqueous		Buoyant-lipid		Particle		Aqueous	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
TG	58.1	7.3	4.7	3.0	3.4	0.8	32.9	1.6	7.3	6.0	18.5	3.4
FFA	nd	–	0.3	0.1	1.2	0.5	nd	–	0.3	0.3	3.1	0.3
ALC	1.0	0.2	nd	–	nd	–	nd	–	nd	–	nd	–
ST	0.9	0.2	0.7	0.2	1.1	0.2	0.4	0.2	1.6	0.6	2.0	0.3
AMPL	2.1	0.4	0.3	0.1	0.5	0.1	1.7	0.2	0.7	0.2	1.3	0.2
PL	0.9	0.4	14.1	5.3	10.5	2.1	1.4	1.0	7.7	1.9	18.6	6.1

Lipid-class abbreviations as in Table 1.

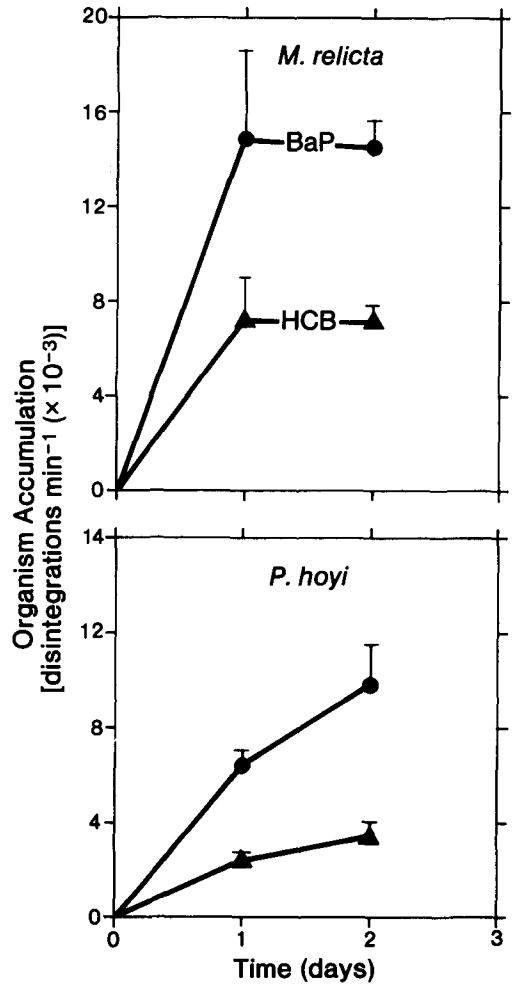

Fig. 4. Relative accumulation of BaP and HCB (as measured by radioactivity) in *M. relicta* and *P. hoyi* during 2 d of exposure in the September experiment. Total accumulation was determined from summing the activities of the three respective experimental fractions (buoyant lipid, particle and aqueous) with that of the residue remaining in the grinding tubes after a portion of the homogenate was removed for lipid fractionation. Each range bar = one SE, based on four or five replicate measurements.

samples were extracted in ethyl acetate after physical partitioning and analyzed by TLC to differentiate between the parent compounds and polar metabolites in each of the fractions (Table 4). A substantial fraction of the ^3H added as BaP was recovered as polar metabolites. The ratio of polar metabolites to parent BaP in the fractions increased as the experiment progressed. The percentage of total ^3H activity measured as polar metabolites ranged from about 50% on day 1 to about 70% on day 4. The rate of biotransformation was 0.225 pmol (g dry wt)$^{-1}$ h^{-1}. Although some ^3H-polar metabolites were found in each of the fractions, the highest concentrations were consistently found in the aqueous fractions (Table 4). In contrast, the highest concentrations of parent BaP were consistently found in the buoyant-lipid fractions. Intermediate levels of both parent BaP and ^3H-polar metabolites occurred in the particle fractions. Thus, although the distribution of total ^3H was dominated by the relatively high concentrations of polar metabolites in *M. relicta*, the *parent* BaP was still distributed approximately in proportion to the total lipid content of the three fractions (Table 4; Fig. 3).

In contrast to the results for BaP, only minimal quantities (<5%) of ^{14}C added as HCB was recovered as polar metabolites (Table 4). The small amount of radioactivity that occurred in the water-soluble fraction could be explained either by slight biotransformation of HCB or by selective binding of a small portion of the parent compound to polar biological materials at the origin of the TLC plate. The latter explanation seems probable because the relative amount of polar metabolites decreased, rather than increased, after the initial day of exposure and because HCB is not easily biotransformed. Biotransformation of HCB, if it occurred at all, was clearly small relative to that of BaP in *M. relicta*.

The parent BaP reached apparent steady state among the lipid pools within 24 h (Table 4). In contrast, HCB required between 24 and 48 h to reach apparent steady state among lipid pools in *M. relicta* (Tables 3 and 4). Reasons for this difference between the two xenobiotics are not clear.

DISCUSSION

Physical fractionation of lipids and contaminants

The described aqueous based method provides a simple and effective way to partially separate energy storage lipids (triacylglycerols) from membrane lipids (phospholipids) in macroinvertebrates. This approach eliminates the problem of having the solvent-extraction process interfere with experimental observations of lipid-contaminant associations that occurred in the animals. The plungers supplied with the Wiretrol pipets are conveniently adapted to extruding both the buoyant-lipid and particle layers from the modified pipet barrels af-

Table 3. Percentages of [^{3}H]benzo[*a*]pyrene and [^{14}C]hexachlorobiphenyl incorporated into the buoyant-lipid (buoy-lipid), particle and aqueous fractions of *Pontoporeia hoyi* and *Mysis relicta* after simultaneous exposure to the two compounds for 1, 2 or 4 d

	P. hoyi						*M. relicta*					
	^{3}H			^{14}C			^{3}H			^{14}C		
	$\bar{X}$	SE	*N*	$\bar{X}$	SE	*N*	$\bar{X}$	SE	*N*	$\bar{X}$	SE	*N*
September experiment												
Day 1												
Buoy-lipid	54.1	2.8	5	47.7	2.9	5	7.1	0.3	4	22.7	2.2	4
Particle	17.5	2.6	5	22.3	3.2	5	33.8	2.8	4	33.5	3.9	4
Aqueous	28.4	1.9	5	29.9	2.5	5	59.1	2.6	4	43.9	3.3	4
Day 2												
Buoy-lipid	57.7	5.8	5	48.4	5.9	5	6.8	1.2	5	46.3	3.2	5
Particle	14.9	2.5	5	20.6	2.3	5	33.2	1.4	5	21.8	0.9	5
Aqueous	27.5	3.8	5	31.0	3.4	5	59.9	1.7	5	31.9	2.8	5
November experiment												
Day 1												
Buoy-lipid							5.6	1.1	6	8.3	2.3	6
Particle							22.7	3.2	6	28.0	2.4	6
Aqueous							71.8	3.8	6	63.6	3.8	6
Day 2												
Buoy-lipid							6.8	0.9	6	27.3	4.4	6
Particle							29.8	2.7	6	26.0	2.6	6
Aqueous							63.4	2.3	6	46.7	3.7	6
Day 4												
Buoy-lipid							6.0	0.6	6	26.6	5.3	6
Particle							27.1	4.2	6	28.8	4.9	6
Aqueous							66.8	4.5	6	44.6	6.8	6

Values given as percentages of the total activity of the isotope summed from the three fractions.

Table 4. Percent distribution of radiotracers as parent compound, polar metabolites or unidentified components (other) in buoyant-lipid (buoy-lipid), particle and aqueous fractions of *M. relicta* sampled at 1, 2 and 4 d of exposure to BaP (^{3}H-labeled) and HCB (^{14}C-labeled) in the November experiment

	^{3}H			^{14}C		
	Parent compound	Polar metabolites	Other	Parent compound	Polar metabolites	Other
Day 1						
Buoy-lipid	25.0	5.3	2.8	19.4	0.5	0.6
Particle	12.1	12.6	1.0	36.2	0.4	nd
Aqueous	10.3	30.9	1.0	38.5	4.7	nd
Day 2						
Buoy-lipid	14.4	7.4	1.3	38.6	nd	nd
Particle	8.8	14.2	1.2	21.2	nd	nd
Aqueous	7.9	43.1	1.6	36.9	0.7	nd
Day 4						
Buoy-lipid	12.7	8.6	1.6	37.8	0.1	nd
Particle	6.2	13.6	1.2	20.9	0.3	nd
Aqueous	6.5	48.0	1.7	38.6	2.3	nd

nd = not detected. Each number represents a mean from duplicate experiments.

ter centrifugation (Fig. 1). The general precision of the physical separations was demonstrated by the rather narrow range of differences observed in distributions of lipids and contaminants among replicate animals. With only a few exceptions, the standard errors of the mean among replicate animals, for each fraction, were less than 5% of the summed total lipids or isotopes (Figs. 2 and 3) in the three fractions. The variance in replicate samples of course represents animal variation as well as experimental precision.

The interpretation of data on "lipid-associated" contaminant concentrations from this method depends on the following assumptions: (a) the contaminant is actively absorbed and subsequently distributed in the organisms within the time scale of the experiment and (b) little or no desorption of the contaminants from the lipids occurs during the few minutes required to process the organisms. The first assumption of active absorption, rather than surface sorption, was supported for *P. hoyi* by data comparing contaminant uptake by dead vs. living organisms [22]; the amount of contaminant accumulated by dead animals was less than 10% of the amount taken up by living organisms. The second assumption is reasonable because bioconcentration factors for nonpolar organic contaminants are high, and elimination rates are low, for both *M. relicta* and *P. hoyi* [10,22].

Distribution and dynamics of BaP and HCB in P. hoyi and M. relicta

The distributions of ^{3}H (from BaP) and ^{14}C (from HCB) closely followed the distribution of total lipids in *P. hoyi*; no selectivity for specific lipid classes was apparent. This observation was consistent both among time intervals within an experiment (Table 3) as well as among different experiments (Fig. 2). Even though the distributions among the fractions differed in the two experiments, the contaminant distributions were essentially identical to the total-lipid distributions in both cases. This result implies that the contaminants were not selectively partitioned into specific classes of lipids in *P. hoyi* but were distributed relatively evenly among the lipid groups. A similar conclusion was reached for contaminant distributions in fish tissues that had been "quantitatively" extracted with chloroform–methanol [12,13].

The distribution of ^{14}C from HCB in *M. relicta* also resembled that of total lipids more than it resembled the distributions of either triacylglycerols or phospholipids (Fig. 3). A group of *M. relicta* with relatively high triacylglycerol content (September experiment) contained more total lipids and ^{14}C in the buoyant-lipid fraction than did those with lower triacylglycerol levels (November experiment). By comparison, *M. relicta* consistently exhibited a distribution pattern for ^{3}H from BaP that was dissimilar from that of the total-lipids (Fig. 3). As was the case for phospholipids, only a small fraction of total ^{3}H from BaP was found in the buoyant-lipid fraction. However, the ^{3}H was more enriched in the aqueous fractions than it was in the particle fractions relative to phospholipids. This result suggests that *M. relicta* either selectively partitions BaP into phospholipids or, alternatively, that it biotransforms BaP into polar metabolites. Thin-layer chromatographic separation of the ^{3}H compounds (Table 4) indicated that the BaP was metabolized, and the observed relatively high concentration of ^{3}H occurring in the aqueous fraction (e.g., as in Fig. 3) can be attributed to the presence of polar BaP metabolites. Although the percent distribution of the polar metabolites among the three fractions resembled that of the phospholipids, our data does not necessarily indicate that the metabolites were incorporated into the phospholipids; as hydrophilic components, both may have simply been dissolved in the aqueous fractions and excluded from the buoyant lipid fraction. In contrast to the results for total ^{3}H activity, the distribution of parent BaP (Table 4) was more like that of the total lipids (Fig. 3). Thus, as was true for *P. hoyi*, the lipophilic parent compound apparently was not selectively partitioned into specific lipid classes in *M. relicta*. However, the hydrophilic metabolites were selectively partitioned into the aqueous and particle fractions. The latter phenomenon was not observed in *P. hoyi* because *P. hoyi* do not measurably biotransform BaP [23].

The similarity of the distributions of both BaP and HCB (Table 3) to that of total lipids in *P. hoyi* (Fig. 1) on both days of the September experiment suggests that both of these compounds reached apparent steady state rapidly among lipid classes in *P. hoyi*. Similar results were observed for parent BaP in *M. relicta* (Table 4), but the kinetics of distribution for HCB were apparently much slower as HCB required about 2 d to reach steady state among the various lipid pools in *M. relicta* (Tables 3 and 4). This delayed distribution of HCB among the different lipid pools of *M. relicta* may indicate part of the mechanism for the relatively slow elimination of HCB that is observed in *M. relicta* [24].

The above comparison of contaminant distributions with lipid-class distributions in *P. hoyi* and *M. relicta* was of particular interest because these

animals exhibit different xenobiotic elimination kinetics and biotransformation patterns for the two classes of important contaminants represented by BaP and HCB. In *M. relicta*, the previously observed rapid elimination of BaP compared to that of HCB [24] was not a result of differential distribution among lipid classes but could be attributed to the relatively rapid biotransformation of the BaP to polar metabolites. In *P. hoyi*, BaP was eliminated faster than HCB by about a factor of 10 [25] but, in contrast to *M. relicta*, *P. hoyi* exhibited no evidence of biotransformation of either the polycyclic aromatic hydrocarbon (BaP) or polychlorinated biphenyls (PCB) [22]. Furthermore, the rate constant for elimination of BaP in *P. hoyi* was approximately the same as the rate of lipid loss and inversely proportional to the total-lipid content. In contrast, no measurable loss of a tetrachlorobiphenyl was observed in *P. hoyi* [22].

Note that the different kinetics for the PCB compared to the PAH in *P. hoyi* may have been due to a differential distribution of the two types of compounds among the various lipid compartments in the organism, but this hypothesis was not supported from the distribution data (Fig. 2). Similar distributions of both HCB and BaP were observed among the different *P. hoyi* lipid compartments. For both compounds, the distribution of the two compounds among the fractions was proportional to the distribution of the total lipids rather than to the specific distributions of either phospholipids or triacylglycerols. We conclude that a mechanism other than selective lipid-class association must be responsible for the differential loss of the two types of contaminants in *P. hoyi*.

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